

What is claimed is:

1. A method for detecting a metabolic disorder in an individual, comprising:

(a) contacting a sample comprising:

5 (i) one or more metabolically indicative enzymes and

(ii) one or more metabolic analytes,

with one or more substrates for said one or more enzymes to produce a reaction admixture, under conditions wherein at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product, 10 and wherein one or more protease inhibitors are present;

(b) contacting said reaction admixture with a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said one or more metabolic analytes and said at least one product are soluble in said reagent; to produce a test sample and

15 (c) determining the presence or amount of said one or more metabolic analytes and said at least one product contained in said test sample using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said 20 metabolic disorder.

2. The method of claim 1, wherein said sample is a body fluid sample.

3. The method of claim 2, wherein said body fluid sample is blood.

25 4. The method of claim 1, wherein said sample is dried.

5. The method of claim 1, wherein said individual is a human suspected of having a metabolic disorder.

30 6. The method of claim 1, wherein said individual is a neonate.

7. The method of claim 1, wherein said individual is a newborn.

8. The method of claim 1, wherein said individual is a child.
9. The method of claim 1, wherein said individual is an adult.
- 5 10. The method of claim 1, wherein said metabolic disorder is an inborn error of metabolism.
11. The method of claim 1, wherein said metabolic disorder is an acquired metabolic disorder.
- 10 12. The method of claim 1, wherein said metabolically indicative enzyme is selected from the group consisting of oxidoreductase, hydrolase, lyase, transferase, ligase and isomerase.
- 15 13. The method of claim 12, wherein the enzyme is a hydrolase.
14. The method of claim 13, wherein the enzyme is a biotinidase.
15. The method of claim 14, wherein said substrate is biocytin.
- 20 16. The method of claim 1, wherein said metabolic analyte is one or more amino acids.
- 25 17. The method of claim 1, wherein said metabolic analyte is an acylcarnitine or plurality of acylcarnitines.
18. The method of claim 1, wherein step (a) further comprises contacting said sample with one or more reference substrates.
- 30 19. The method of claim 1, wherein step (b) further comprises contacting said sample with one or more reference products.

20. The method of claim 1, wherein step (d) further comprises, prior to determining, adding one or more reference products corresponding to the at least one product .

5 21. The method of claim 1, wherein step (d) further comprises, prior to determining, adding one or more reference analytes corresponding to the one or more metabolic analytes contained in said sample.

22. The method of claim 1, wherein said reaction admixture is aqueous.

10 23. The method of claim 22, wherein said reagent is non-aqueous.

24. The method of claim 23, wherein said reagent comprises an organic solvent.

15 25. The method of claim 23, wherein said reagent comprises an alcohol.

26. The method of claim 25, wherein said reagent is methanol.

20 27. The method of claim 1, wherein said mass spectrometry is tandem mass spectrometry.

28. The method of claim 1, wherein one of said protease inhibitors is COMPLETE protease cocktail.

25 29. The method of claim 1, wherein one of said protease inhibitors is PEPSTATIN.

30. A method for detecting a metabolic disorder in an individual, comprising:

(a) contacting a vessel containing one or more substrates in dried form with a sample from said individual, the sample containing

- 5 (i) one or more metabolically indicative enzymes and
(ii) one or more metabolic analytes,
in aqueous solution;

under conditions wherein at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product, and wherein one or
10 more protease inhibitors are present;

(b) adding a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said one or more metabolic analytes and at least one product are soluble in said reagent;

15 (c) determining the presence or amount of said one or more metabolic analytes and said at least one product in the resultant mixture using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.

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31. The method of claim 30, wherein said vessel further contains one or more reference substrates.

25 32. The method of claim 30, wherein said vessel further contains one or more reference analytes corresponding to the one or more metabolic analytes in said sample.

33. The method of claim 30, wherein said vessel further contains one or more reference products.

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34. The method of claim 30, wherein one of said protease inhibitors is COMPLETE protease cocktail.

36. The method of claim 30, wherein one of said protease inhibitors is PEPSTATIN.

37. A method for detecting a metabolic disorder in an individual,
5 comprising:

- (a) contacting a sample comprising:
 - (i) one or more metabolically indicative enzymes and
 - (ii) one or more metabolic analytes,

with one or more substrates for said one or more enzymes to produce a
10 reaction admixture, under conditions wherein at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product, and wherein one or more protease inhibitors are present;

(b) contacting said reaction admixture with a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein
15 said one or more metabolic analytes and said at least one product are soluble in said reagent, to produce a test sample;

(c) contacting said reaction admixture with one or more reference products and one or more reference analytes, and

(d) determining the presence or amount of said one or more metabolic
20 analytes and said at least one product contained in said test sample, with respect to said one or more reference products and one or more reference analytes, using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said
25 metabolic disorder.

38. A method for detecting a metabolic disorder in an individual, comprising:

(a) separating a sample into two sample portions, the sample comprising:

- 5 (i) one or more metabolically indicative enzymes and
(ii) one or more metabolic analytes;

(b) contacting each sample portion with one or more substrates for said one or more enzymes to produce two reaction admixtures, under conditions wherein at least one of said enzymes in each sample portion is capable of acting
10 on a corresponding substrate to generate at least one product, and wherein one or more protease inhibitors are present in at least one of said reaction admixtures;

(c) contacting each reaction admixture with a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said reagent can be the same or different for each reaction admixture, and wherein
15 said one or more metabolic analytes and said at least one product are soluble in said reagent or reagents; to produce a test sample and

(d) combining the two reaction admixtures to produce a test sample, and
(e) determining the presence or amount of said one or more metabolic analytes and said at least one product contained in said test sample using mass
20 spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.

39. A method for detecting a metabolic disorder in an individual, comprising:

(a) separating a sample into two sample portions, the sample comprising:

- 5 (i) one or more metabolically indicative enzymes and
(ii) one or more metabolic analytes;

(b) contacting each sample portion with individual vessels, each vessel containing one or more substrates for said one or more enzymes in dried form,

10 (c) contacting each vessel with a solution to produce two reaction admixtures, under conditions wherein at least one of said enzymes in each sample portion is capable of acting on a corresponding substrate to generate at least one product, and wherein one or more protease inhibitors are present in at least one of said reaction admixtures;

(d) contacting each reaction admixture with a reagent that inhibits the 15 ability of said one or more enzymes to act on a corresponding substrate, wherein said reagent can be the same or different for each reaction admixture, and wherein said one or more metabolic analytes and said at least one product are soluble in said reagent or reagents; to produce a test sample and

20 (e) combining the two reaction admixtures to produce a test sample, and
(f) determining the presence or amount of said one or more metabolic analytes and said at least one product contained in said test sample using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said 25 metabolic disorder.

40. A method for detecting a metabolic disorder in an individual, comprising:

(a) contacting a vessel containing one or more substrates in dried form with a sample from said individual, the sample containing

- 5 (i) one or more metabolically indicative enzymes and
(ii) one or more metabolic analytes,

(b) contacting said vessel with a solution to generate conditions wherein at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product, and wherein one or more protease inhibitors are
10 present;

(c) adding a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said one or more metabolic analytes and at least one product are soluble in said reagent;

15 (d) determining the presence or amount of said one or more metabolic analytes and said at least one product in the resultant mixture using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.